Claim 19 Support Chart		
	Claim Limitations	**Support in the Application**
I	A method of injecting a defined volume of sample into an electrolyte channel in a microfluidics device, comprising	"Thus, the sample-filled part 27 of the channel piece of the sampling devices 3 defines the volume of the electro-kinetically injected sample plug..." Page 6, lines 33-35.
II	placing a sample in a sample channel that intersects the electrolyte channel at a supply port,	"The source channel S and the drain channel D each discharge into the channel piece 22 at respective supply and drain ports 25, 26." Page 6, lines 10-11.
III	injecting sample in the sample channel along a pathway that includes the supply port, a drain port intersecting the electrolyte channel at location axially spaced from the first port, and a segment of the electrolyte channel between the two ports, where the sample volume is defined as the region of the electrolyte channel extending between and along the two ports,	"Preferably the supply port 15 and the drain port 16 are spaced apart from each other at the channel piece 12 and delimit a sample volume 17. The distance d which they are spaced apart from each other typically amounts to from about 0 μm to about 3 cm, most preferably to about 3 mm, wherein the value 0 indicates that the supply and drain ports are located opposite each other." Page 9, lines 9-13.
IV	by applying an electric field across the supply and drain channels,	"After the channel system of the chemical analysis system has been filled with the electrolyte buffer, the injection of the sample into the channel piece 22 is initiated. For that purpose an electric field is established between the source receptacle S and the drain receptacle D..." Page 7, lines 19-22.
V	by said injecting, producing a defined sample volume in the electrolyte channel, and	"Thus, the sample-filled part 27 of the channel piece of the sampling devices 3 defines the volume of the electro-kinetically injected sample plug..." Page 6, lines 33-35.
VI	electrokinetically moving the defined sample volume along the electrolyte channel by applying an electric field across a reservoir for the electrolyte buffer and a drain at an opposite end of the electrolyte channel	"For that purpose an electric field is established between the source receptacle S and the drain receptacle D such, that the sample is electro-kinetically transported from the source receptacle S through the supply channel 23 via the channel piece 22 into the drain channel 24 and on to the drain receptacle D." Page 7, lines 21-25.

Support for new Claim 20 is set forth in the following table.

Support for Claim 20		
	Claim Limitations	**Support in the Application**
I	wherein, during said moving, subjecting said supply and drain channels to an electric potential which is different from the electric potential at the reservoir for the electrolyte buffer, thus establishing a potential difference such that the electrolyte buffer is allowed to advance into said supply channel and into said drain channel.	"In order to allow the electrolyte buffer to advance into the supply and drain channels 23 and 24, in the exemplary embodiment of the sampling device depicted in Fig. 3 the source receptacle S and the drain receptacle D are switched on an electric potential which is different from the electric potential at the reservoir R for the electrolyte buffer, thus establishing a potential difference of suitable magnitude." Page 8, lines 23-27.

Support for new Claim 21 is set forth in the following table.

Claim 21 Support Chart		
	Claim Limitations	**Support in the Application**
I	wherein said potential difference is chosen such that a resultant electric field strength amounts to at least about 0.1 V/cm.	"Preferably the potential difference between the reservoir R and the source and drain receptacles S, D is chosen such, that the resultant electric field has a field strength which amounts to at least about 0.1 V/cm." Page 8, lines 34-36.

Identified support in the specification for new Claims 19-21 can be found in the above tables. Applicants believe such claims are fully supported by the specification and, therefore, do not introduce new matter.

II. Patentability of the claimed invention

The following remarks are made pursuant to Section 7.08.02, VIIIE of the MPEP, which requires the applicant to submit a detailed discussion of the references deemed most closely related to the subject matter of the present invention, pointing out how the claimed subject matter is patentable over the references. The references discussed are those cited during prosecution of related U.S. Patent application Serial Number 08/226,605, filed April 12, 1994, which this application claims priority to. Copies of the references were previously submitted in the priority application.

A. Advantages and features of the invention

The claimed method provides the principle feature that a known amount of a controlled, geometrically defined sample volume can be electrokinetically introduced in microcolumn separation techniques. This feature has important advantages in that (i) predictable amounts of sample components are injected, resulting in low background noise of the detected signal, thus increasing the limits of detection considerably, and (ii) because the volume of the sample is known, internal standards do not have to be used for quantitative analysis.

B. Patentability over the prior art

The ability to achieve controlled sample introduction, in accordance with the presently claimed invention, is unsuggested in the prior art for the reasons given below. The conclusions about the scope and content of the prior art, discussed below, are made on the basis of a review of the prior art cited in the enclosed Information Disclosure Statements, particularly art cited during the prosecution in U.S. patent application Serial

No. 08/226,605, and a pre-examination search conducted by an authorized agent.

1. There is no evidence in the prior art of how to provide a geometrically defined sample in an electrophoresis device.

Although the prior art appears to recognize that the sample should be clearly defined, there is no prior art recognition of how to provide a geometrically defined sample in an electrophoresis device as claimed in Claim 19.

As discussed in Section C below, Deml *et al.*, Harrison I, Byers *et al.*, and Pace *et al.* describe devices that lack either a supply channel or a drain channel or both which intersects the electrolyte channel. Thus, these devices are unable to provide a geometrically defined sample volume. Although Verheggen *et al.* includes both a supply and drain channel that intersects the electrolyte channel, and Harrison II describes a device that has two channels that intersect, both Verheggen *et al.* and Harrison II fail to recognize the specific benefit of being able to define the sample volume by manipulating the intersection of the channels.

2. The prior art teaches away from the use of electokinetic injection to introduce the sample.

The present method requires that a sample be electrokinetically injected into the electrolyte channel of the device whereby the sample volume is geometrically defined by a section of the electrolyte channel located between a supply channel and a drain channel.

The claimed method is distinguished from the prior art in that it requires that a sample be electrokinetically injected into the electrolyte channel. Verheggen *et al.* teaches against the use of an electrokinetic technique to introduce the sample

because such electrokinetic techniques do not result in the introduction of representative sample aliquots. Thus, although Verheggen *et al.* recognizes the problem solved by the claimed invention, the reference offers no solution to the problem.

C. Discussion of cited references

The following references were cited during prosecution of related U.S. patent application Serial Number 08/226,605, filed April 12, 1994. Applicant submits that the presently claimed invention patentably defines over these references for the following reasons:

1. Deml *et al.* (1985) *Journal of Chromatography* 320: 159-165 describes a method for reproducible sampling of small quantities of sample for high-performance capillary electrophoresis. The method is based on the principle of the splitter. The sample migrates electrophoretically in two electrical circuits and the splitting ratio is given by the ratio of the corresponding electric currents. However, there is no supply channel intersecting an electrolyte channel at a supply port. Thus, the sample volume cannot be defined in the electrolyte channel between two ports.

2. Harrison *et al.* (1992) *Analytical Chemistry* 64: 1927-1932 ("Harrison I") describes a microfabricated electrophoresis device having a sample-injector formed by the intersection of two channels (Fig. 1). The sample is injected by dipping one end of a capillary into the sample reservoir and applying a voltage across the ends of the capillary. In the electric field the sample is transported electrokinetically and is injected at a T-junction into the channel system of the capillary electrophoretic device.

In operation a voltage is applied across a sample reservoir 2 and separation reservoir 3 for a brief period, to draw the selected volume sample plug into the separation channel. A voltage is then applied across a mobile-phase reservoir 1 and reservoir 3 at the downstream side of the separation channel, to move sample at the intersection and in the separation channel down the separation channel past a detector.

The sample volume in Harrison I is not defined geometrically. Instead, it is determined by the strength and time of the applied injection voltage. Thus, the sample volume according to Harrison I is not defined by a section of the electrolyte channel located between the supply port and the drain port, as is required by present Claim 19.

3. Harrison *et al.* (1993) *Sensors and Actuators* 107-116 ("Harrison II") discloses a microchannel device having first and second channels crossing at a point to form an intersection and connecting first and second reservoirs (channel 1) and third and fourth reservoirs (channel 2). Sample injection is achieved by drawing sample through the intersection by applying a voltage across reservoirs 1 and 2, followed by switching the applied potentials to reservoirs 3 and 4.

Harrison II does not, however, describe offsetting supply and drain channels along the electrolyte channel. Thus, the sample volume according to Harrison II is not defined by a section of the elctrolyte channel located between the supply port and the drain port, as is required by present claim 19.

4. Verheggen *et al.* (1988) *Journal of Chromatography*, 452:615-622 discloses a sampling device for capillary isotachophoresis and capillary zone electrophoresis whereby the most essential feature of this device is the direct introduction

of the sample solution into a part of the capillary tube by means of two feeders which extend perpendicular to the capillary tube. The arrangement of the two feeders off-set from each other along the longitudinal extension of the capillary tube is such that the sampling device has the shape of a capillary double T structure.

The device described in Verheggen *et al.* is distinguished from the claimed device in two aspects. First, Verheggen *et al.* fails to recognize the benefit of manipulating the supply and drain channel intersections with the electrolyte channel to form a geometrically defined sample volume between the supply and drain ports.

Second, Verheggen *et al.*, at page 622, advises against the use of an electrokinetic technique to introduce the sample because such electrokinetic techniques do not result in the introduction of representative sample aliquots. Indeed, nearly the entire first paragraph of page 622 is devoted to discussing the disadvantages of using electrokinetic sample introduction in the disclosed apparatus. For example, the last three sentences describe an experiment where sampling was carried out with an electromigration technique. However, the experiment supported the conclusion that electrokinetic sampling techniques should not be used because the technique failed to introduce representative sample aliquots. Therefore, although Verheggen *et al.* recognizes the problem solved by the claimed invention, the reference offers no solution to the problem.

5. Byers *et al.* (US Pat. No. 4,941,958) describes a device having a first end, a second end, a first ion exchange medium extending from the first end to the second end, and a second ion exchange medium, which is in fluid communication with and has higher ion mobility than the first medium. The device, however,

does not contain supply and drain channels that intersect an electrolyte channel. Thus, the device is unable to define a sample volume.

6. Pace *et al.* (US Pat. No. 4,908,112) describes an analytical separation device in which a capillary sized conduit is formed by a channel in a semiconductor device and the channel is closed by a glass plate. Electrodes are positioned in the channel to activate the motion of liquids through the conduit by electroosmosis.

Although the device described in Pace *et al.* includes a separation conduit and a sample channel, it lacks a drain channel. Without a drain channel to intersect the separation conduit, the sample volume cannot be defined geometrically.

Respectfully submitted,

Larry Thrower

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Larry W. Thrower
Registration No. P47,994

CORRESPONDENCE ADDRESS
Customer No. 22918
(650) 324-0880